Column E Explanation for Registration Number 93-R-0219

1. Number of Animals used in this study: **25**
2. Species of animals used in this study: **Ferret**
3. Explanation of the procedure producing pain and/or distress: **Animals are infected with influenza.**
4. Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that the pain and/or distress relief would interfere with test results.

   During the study, it is not possible to provide additional pain or distress relief such as analgesics. The infection results in inflammation and a cytokine response which are associated with the symptoms we are measuring to determine research therapy efficacy. Any indirect impact on the symptoms would therefore compromise the study goal. For that reason, some animals may experience discomfort and this would include vehicle treated infection control animals and animals that receive an ineffective dose, i.e. a treatment that is only partially protective. However, the strains we have typically used in this model appear to cause limited discomfort and in our experience no animal has ever needed to be preemptively euthanized on the basis of clinical signs. Symptoms, when present, should peak typically 48-72 hours after infection and will completely resolve as the animal clears the infection.

5. What, if any, federal regulations require this procedure? **NA**

1. Number of Animals used in this study: **550**
2. Species of animals used in this study: **Hamster**
3. Explanation of the procedure producing pain and/or distress: **Animals are infected with C. difficile.**
4. Scientific justification why pain and/or distress could not be relieved. State methods or means used to determine that the pain and/or distress relief would interfere with test results.

   During the acute phase of the model, disease progression can be rapid, especially in the vehicle treated (infection control) group. For this reason a monitoring system is utilized where animals are scored up to every 30-60 minutes, with animals removed from study if they reach a clinical score of 2 or below. During the relapse phase of the model, symptoms of disease can also be observed, but at a much slower rate. Death is not an endpoint in this model and pain and distress will be relieved by the use of humane endpoints.

   It is not possible to use chemical means of pain relief, as antibiotics have been shown to interact with many other classes of drug and so co-treatment may compromise the study goal, which is the direct investigation of antibiotic/microbe interaction. Co-administration of antibiotics and non-steroid anti-inflammatory drugs have been shown to cause adverse drug reactions clinically and also in *in vivo* studies. For example quinolones have been shown to induce convulsions in mice when used in conjunction with anti-inflammatory drugs. Furthermore, the use of analgesics could interfere with infection progression and the activity of an antibiotic due to anti-inflammatory and inherent antimicrobial activity. Analgesics such as morphine have been shown to have antibacterial effects, either alone or by acting synergistically with antibiotics. As we will be testing investigational compounds, it is unknown what effect the drug interaction will have. A synergistic effect may result in an overstatement of the antibacterial effect, while an antagonistic effect will mean that the full potential of the antibacterial is not observed.

5. What, if any, federal regulations require this procedure? **NA**